"Preliminary Note on the Resistance to Heat of *B. anthracis*." By A. Mallock, F.R.S., and Lieut.-Col. A. M. Davies, R.A.M.C. Received November 30,—Read December 10, 1903.

Very discrepant statements have been made by various authorities as to the degree of temperature and duration of heating requisite to destroy the spores of various species of bacteria. The great importance of the subject, especially as regards the rapid and effective sterilisation of water for troops in the field, made us think it worth while to undertake the experiments here recorded.

Our object was to determine a curve whose ordinates should represent the time necessary for sterilisation in terms of the temperature to which the infected water was heated. Since many of the authorities give very long times as necessary (ranging from 10 minutes to some hours) even at temperatures considerably above 100° C., we expected to find that the slope of the curve would be a comparatively gentle one, and would meet the temperature axis at a finite but acute angle. This expectation, however, was not fulfilled, and we rarely found any survival of living matter in fluid which had been raised to a temperature of 100° C. even for as short a time as 20—30 seconds.

In our earlier experiments (Nos. 1—85) we used *B. anthracis*, *B.m. ruber*, *Staph. p. aureus*, *Staph. p. citreus* and *B. subtilis*, but subsequently we confined our attention to anthrax, and the results here recorded refer only to this latter, which may be considered as a typically resistant germ.

The experiments consisted in exposing infected water in sealed glass tubes to heat in steam, at various temperatures, and for various times, the contents of the tubes being afterwards incubated in broth.

The method and procedure employed is described in some detail, so that there may be no doubt about the conditions under which the results were obtained.

The heat was applied in a small closed chamber (A) (fig. 1), which by means of a two-way cock (B) could be placed in communication either with a steam boiler, or with the outer air.

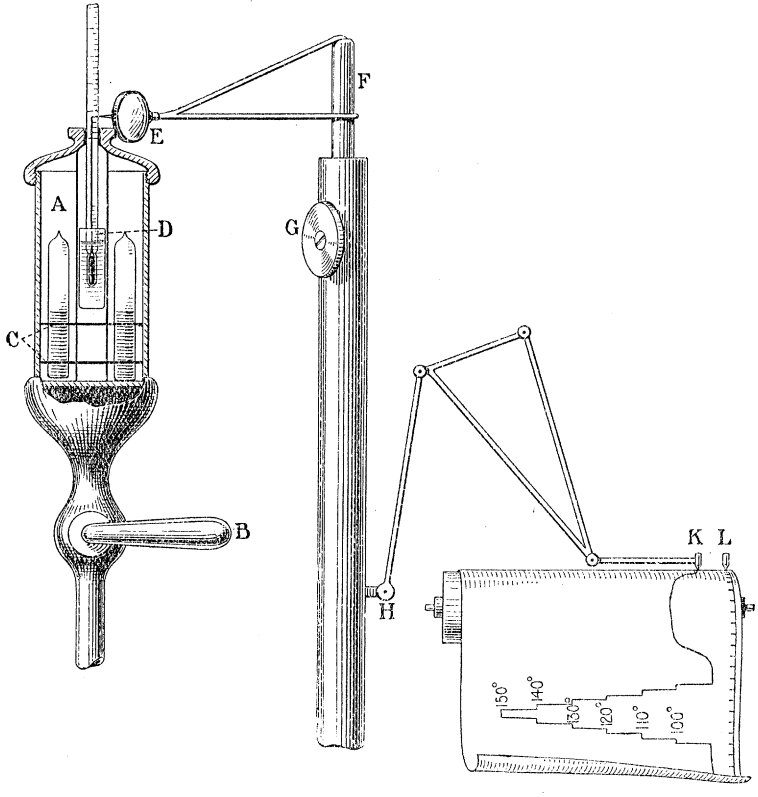
The chamber had an easily removable steam-tight cover, to which was attached a light brass holder (C) fitted to receive six of the culture tubes.

Through the centre of the cover a thermometer was introduced into the chamber, its stem passing through a steam-tight packing, and its bulb dipping into water contained in the glass tube (D), of the same size as the culture tubes. The stem of the thermometer outside the chamber could be viewed through a lens attached to a movable pointer (E). This pointer was carried on the vertical rod (F), which could be moved up and down by the milled head (G), and a pen (K), connected

by levers with the rod at (H), marking on uniformly moving paper, indicated the position of the pointer at each instant. Time was also recorded on the paper by means of another pen (L) worked from an electric clock beating seconds.

During an experiment the pointer was kept carefully on the end of the mercury column in the thermometer, so that the diagram on the paper gave the temperature of the tubes in terms of time.

Fig. 1.



The tube in which the thermometer bulb dipped had the same amount of water in it, to start with, as was contained in the culture tubes, but as the thermometer tube was open to the steam in the chamber, and the culture tubes were sealed, the water in the former would acquire the temperature of the steam more rapidly than the liquid in the culture tubes. Thus the temperature to which the cultures were exposed must have been a little less than that recorded, but only when the times of heating were very short would the difference be appreciable.

The actual procedure in all the experiments was as follows:—Sterilised glass tubes 3 inches long and $\frac{1}{2}$ inch diameter were about half filled with infected water, and the tubes were then sealed with the blow-pipe. This was effected without warming the contents by placing the tubes upright in a holder revolved by clockwork at about thirty revolutions per minute; the top of the tube was then heated by an upward pointing flame until its diameter contracted to about $\frac{1}{4}$ inch, and drawn to a point by means of a previously heated glass tube held in the hand.

The tubes were next placed in the holder attached to the cover of the chamber, the water in the tube surrounding the thermometer was brought to the same temperature as the sealed tubes and the cover placed on the heating chamber. Before admitting steam, however, the paper record was started and the pointer (E) brought successively to 90°, 100°, 110°, 120°, 130°, 140°, 150°, on the thermometer scale, so that each record might have on it a thermometer scale for reference. Steam was then admitted and the requisite temperature maintained for the time determined on. With a little practice in manipulating the two-way cock it was found easy to keep the temperature constant within a quarter of a degree Centigrade. Of course the temperature which it was wished to maintain was settled beforehand, but in the results the temperatures are taken from the records.

The method of inoculating the tubes was as follows:—The tubes, having been plugged and sterilised by dry heat, were half filled with distilled water, re-plugged, and then sterilised in the steamer for half an hour on three successive occasions. On the morning of the day of experiment the tubes were inoculated with the respective organisms, either from an agar or broth culture, as stated in each instance, and again plugged. Within two or three hours the inoculated tubes were sealed up and submitted to the different degrees and durations of heat, as detailed in the table. This having been done, the tops of the tubes were filed off, and the contents sown into broth with the least possible delay, generally within two hours. Microscopic examination of the culture inoculated was made in each case immediately before sowing into the tubes; and afterwards, as soon as growth (if any) made its appearance in the inoculated broth, in order to verify the purity of the cultures.

Care was taken in all cases to be sure that spores were present in the infected water.

The original growth of *Bacillus anthracis* was obtained from a pure culture on agar, supplied by the kindness of Dr. J. W. H. Eyre, Bacteriologist to Guy's Hospital; this was derived from the blood of a fatal case of anthrax in the wards of the Hospital in March, 1903. Sub-cultures were made from this strain, and inoculated into the tubes of water, as detailed in the tables.

The following appearances were relied on, as indicating the growths of *B. anthracis*:—

Nutrient Broth at 37° *C.*—After 24—48 hours, whitish deposit, and presence of small flocculent masses in upper part of tube, which fall down on shaking; the broth itself remains clear; absence of any pellicle.

Agar Stroke at 37° *C.*—Whitish thin defined growth along the stroke, with irregular edges, not spreading widely.

Microscopic Appearances.—Rods, threads and felted masses, with spores either free or lying within the rods. Rods non-motile.

The subjoined table gives the results of all the experiments made on anthrax.

Experiments on *B. anthracis*.

Date.	Series.	Exp.	Culture.		Heated.		Results after incubation at 37°.
			In.	Age of.	To.	For.	
1903.				days.	°C.	m. s.	
June 11	I	1	A*	3	104·5	5 0	No growth.
		2	B†	2	104·5	5 0	,,
,, 16	II	6	A	7	104·5	5 0	,,
		7	B	6	104·5	5 0	Contaminated growth.
		8	B	3	104·5	5 0	Some growth.
,, 19	III	13	B	6	110·0	5 0	No growth.
		14	B	6	110·0	5 0	Contaminated.
,, 19	IV	19	B	6	115·0	5 0	No growth.
		20	B	3	115·5	5 0	,,
,, 26	V	25	A	2	110·5	5 0	Contaminated.
		26	B	2	110·5	5 0	No growth.
,, 26	VI	31	A	2	110·5	2 30	,,
		32	B	2	110·5	2 30	,,
July 2	VII	37	A	8	110·5	2 30	,,
		38	B	8	110·5	2 30	,,
,, 2	VIII	43	A	8	115·5	2 30	,,
		44	B	8	115·5	2 30	,,
,, 15	IX	49	A	21	115·5	2 30	,,
		50	B	36	115·0	2 30	,,
,, 15	X	55	A	21	117·0	1 0	,,
		56	B	36	117·0	1 0	,,
,, 22	XI	61	A	27	118·0	1 0	,,
		62	B	42	118·0	1 0	,,
,, 22	XII	67	A	27	116·0	0 30	,,
		68	B	42	116·0	0 30	,,
,, 29	XIII	73	A	35	116·0	0 30	,,
		74	B	50	116·0	0 30	,,
,, 29	XIV	79	A	35	114·0	0 20	,,
		80	B	50	112·5	1 0	,,
Aug. 12	XV	85	B	29	112·5	1 0	,,
		86	B	29	112·5	1 0	,,
,, 12	XVI	87	B	29	111·0	2 0	,,
		88	B	29	111·0	2 0	,,

* A stands for agar. † B for broth.

Date.	Series.	Exp.	Culture.		Heated.		Results after incubation at 37°.
			In.	Age of.	To.	For.	
1903.				days.	°C.	m. s.	
Aug. 12	XVII	89	B	29	114·0	0 10	No growth.
					107·0	0 50	
		90	B	29	114·0	0 10	,,
					107·0	0 50	
,, 12	XVIII	91	B	29	107·0	1 0	,,
		92	B	29	107·0	1 0	,,
,, 12	XIX	93	B	29	104·0	1 0	,,
		94	B	29	104·0	1 0	,,
,, 12	XX	95	B	29	102·0	1 0	,,
		96	B	29	102·0	1 0	,,
,, 18	XXI	97	B	35	110·5	0 20	Slight growth.
		98	B	35	110·5	0 20	No growth.
,, 18	XXII	90	B	35	101·5	0 20	,,
		100	B	35	101·5	0 20	,,
,, 18	XXIII	101	B	35	101·5	0 12	,,
		102	B	35	101·5	0 12	,,
,, 18	XXIV	103	B	35	100·0	1 0	,,
		104	B	35	100·0	1 0	,,
,, 18	XXV	105	B	35	100·0	0 20	,,
		106	B	35	100·0	0 20	,,
,, 18	XXVI	107	B	35	100·0	2 0	,,
		108	B	35	100·0	2 0	,,
Sept. 24	XXVII	109	B	6	99·0	5 0	Slight growth.
		110	B	6	99·0	5 0	No growth.
,, 24	XXVIII	111	B	6	99·0	4 0	,,
		112	B	6	99·0	4 0	,,
,, 24	XXIX	113	B	6	99·0	3 0	,,
		114	B	6	99·0	3 0	,,
,, 24	XXX	115	B	6	100·0	5 0	,,
		116	B	6	100·0	5 0	,,
,, 24	XXXI	117	B	6	100·0	2 0	,,
		118	B	6	100·0	2 0	,,
,, 24	XXXII	119	B	6	100·0	1 0	,,
		120	B	6	100·0	1 0	,,
,, 30	XXXIII	121	A	45	Not	heated	Copious growth, contaminated.
		122	A	45	,,	,,	Copious growth of anthrax.
,, 30	XXXIV	123	A	45	100·0	5 0	No growth.
		124	A	45	100·0	5 0	,,
,, 30	XXXV	125	A	45	100·0	2 30	Copious growth, not anthrax.
		126	A	45	100·0	2 30	Contaminated growth.
,, 30	XXXVI	127	A	45	100·0	1 0	Copious growth, contaminated.
		128	A	45	100·0	1 0	,, ,,
,, 30	XXXVII	129	A	45	99·0	5 0	,, ,,
		130	A	45	99·0	5 0	,, ,,
,, 30	XXXVIII	131	A	45	99·0	2 30	,, ,,
		132	A	45	99·0	2 30	,, ,,
Oct. 7	XXXIX	133	A	52	101·0	2 30	No growth.
		134	A	52	101·0	2 30	Contaminated.
,, 7	XL	135	A	52	101·0	1 0	,,
		136	A	52	101·0	1 0	No growth.

Date.	Series.	Exp.	Culture.		Heated.		Results after incubation at 37°.
			In.	Age of.	To.	For.	
1903.				days.	°C.	m. s.	
Oct. 7	XLI	137	A	52	101·0	1 0	No growth.
		138	A	52	101·0	1 0	,,
,, 7	XLII	139	A	52	105·0	1 0	Contaminated.
		140	A	52	105·0	1 0	No growth.
,, 7	XLIII	141	A	52	105·0	0 30	Contaminated.
		142	A	52	105·0	0 30	No growth.
,, 7	XLIV	143	A	52	105·0	0 12	Contaminated.
		144	A	52	105·0	0 12	No growth.
,, 14	XLV	145	A	5	100·0	2 30	,,
		146	A	5	100·0	2 30	,,
,, 14	XLVI	147	A	5	100·0	1 0	,,
		148	A	5	100·0	1 0	,,
,, 14	XLVII	149	A	5	100·0	0 20	,,
		150	A	5	100·0	0 30	,,
,, 14	XLVIII	151	A	5	101·0	2 30	,,
		152	A	5	101·0	2 30	,,
,, 14	XLIX	153	A	5	101·0	1 0	,,
		154	A	5	101·0	1 0	,,
,, 14	L	155	A	5	103·0	0 30	,,
		156	A	5	103·0	0 30	,,
,, 22	LI	157	A	8	99·5	0 20	,,
		158	A	8	99·5	0 20	,,
,, 22	LII	159	A	8	101·0	0 30	,,
		160	A	8	101·0	0 30	,,
,, 22	LIII	161	A	8	99·8	10 0	,,
		162	A	8	99·8	10 0	,,
,, 22	LIV	163	A	8	99·5	5 0	,,
		164	A	8	99·5	5 0	,,
,, 22	LV	165	A	8	102·0	0 30	,,
		166	A	8	102·0	0 30	,,
,, 22	LVI	167	A	8	103·0	0 25	,,
		168	A	8	103·0	0 25	,,

It will be seen that in all 113 experiments were made; of these 95 were at temperatures above 100° C. and 18 below.

Out of the 95, 14 cases occurred in which some growth took place after incubation. In 12 cases out of the 14 the growth was contaminated.

Out of the 18 experiments below 100° C., growth occurred in 5 cases, in only 1 of which (viz., in Experiment 109) was a pure Anthrax developed.

Whether Anthrax was really present in any of the contaminated growths is somewhat doubtful. All that can be said is that in 3 cases the usual Subtilis contamination was accompanied by a non-motile spore-bearing bacillus growing in threads, which, as far as appearance goes, might be Anthrax.

Looking at the experiments as a whole, and considering that ten

different sub-cultures were experimented with at many different ages, and that in only two cases (viz., Experiments 8 and 97) was a pure Anthrax growth obtained when the infected water had been raised to 100° C., we conclude that any heating of Anthrax spores in water to this or any higher temperature, even for the shortest practicable time, is almost certain to insure their destruction.

What is the lower limit of destructive temperature, when the heating is prolonged, we have not attempted to determine, but we hope to make some observations on this point both with regard to Anthrax and some other spore-bearing bacilli, and to give the results in a further communication.

"On the Resemblances Exhibited between the Cells of Malignant Growths in Man and those of Normal Reproductive Tissues."* By J. Bretland Farmer, F.R.S., J. E. S. Moore, F.L.S., and C. E. Walker. Received December 8,—Read December 10, 1903.

The object of this communication is to draw attention to certain important cytological transformations exhibited during the development of malignant growths in man. We believe that the changes we are about to describe are diagnostic of malignant as opposed to those of a benign character. Furthermore, if our conclusions are well founded, they may at the same time serve to throw light upon the nature of the processes involved in the formation of these growths, and we hope that they may also serve as a point of departure for further investigations on the more remote ætiology of the disease itself.

We wish, however, at the outset, to disclaim all intention of formulating at the present time any theory as to the nature of these various remote causes, although, as will be seen in the sequel, our observations indicate certain directions along which such causes may perhaps be profitably sought.

We may at once state as the results of our investigations on a large number of malignant growths, including numerous examples of *Carcinomata* and *Sarcomata*, that we have been able to trace in detail a number of definite and serial changes in the cells of the invading and proliferating malignant tissue, which are remarkably similar to those obtaining during the maturation of the elements contained within the sexual reproductive glands, and it would seem that such a resemblance,

* We desire to state that whilst working together at this subject we have each approached the problems from an independent standpoint. The paper is in every sense a joint one.

FIG. 1.